

# Genome-wide identification of QTL for age at puberty in gilts using a large intercross F<sub>2</sub> population between White Duroc × Erhualian

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**Abstract** – Puberty is a fundamental development process experienced by all reproductively competent adults, yet the specific factors regulating age at puberty remain elusive in pigs. In this study, we performed a genome scan to identify quantitative trait loci (QTL) affecting age at puberty in gilts using a White Duroc × Erhualian intercross. A total of 183 microsatellites covering 19 porcine chromosomes were genotyped in 454 F<sub>2</sub> gilts and their parents and grandparents in the White Duroc × Erhualian intercross. A linear regression method was used to map QTL for age at puberty *via* QTLeXpress. One 1% genome-wise significant QTL and one 0.1% genome-wise significant QTL were detected at 114 cM (centimorgan) on SSC1 and at 54 cM on SSC7, respectively. Moreover, two suggestive QTL were found on SSC8 and SSC17, respectively. This study confirmed the QTL for age at puberty previously identified on SSC1, 7 and 8, and reports for the first time a QTL for age at puberty in gilts on SSC17. Interestingly, the Chinese Erhualian alleles were not systematically favourable for younger age at puberty.

**age at puberty / quantitative trait loci / White Duroc / Erhualian**

## 1. INTRODUCTION

Puberty in females is defined as the time of the first expressed oestrus with ovulation, which is a critical development process marking the transition into adulthood. Although the timing of the onset of puberty is influenced by environmental factors, evidence has accumulated that at least 50% of the phenotypic variance of age at puberty is genetically determined in humans [29]. However,

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to date, the specific genetic determinants regulating the maturation of the hypothalamic-pituitary-gonadal axis remain largely unknown. Thus, the identification of determinant factors of age at puberty will improve our understanding of pubertal development and pathophysiology of many reproductive endocrine pathways in mammals.

In pig production, the rate of pubertal development and successful pregnancy in gilts affect the efficient management of breeding females. Selection for growth rate and leanness in modern commercial pigs has resulted in delay in the onset of puberty [23]. Because ages at puberty and weaning to oestrus interval are positively correlated [27] and the primary reason for culling sows is failure to return to oestrus after weaning; selection for younger pigs at puberty has a favourable effect on sow reproductivity [13]. Although the average heritability estimated for puberty age in gilts is 0.32 [25], ages at puberty and oestrus cycles are very laborious to measure. Thus, marker-assisted selection (MAS) may provide a practical and efficient method to improve age at puberty in gilts.

As a first step to identify the gene(s) underlying age at puberty, several genome scans have been performed to identify quantitative trait loci (QTL) for age at puberty. Rohrer *et al.* [24] reported the first two QTL for age at puberty on pig chromosomes (SSC) 1 and 10 in a multigenerational Meishan-White composite resource population. Subsequently, Cassady *et al.* [4] and Holl *et al.* [14] found several QTL for age at puberty on SSC7, 8, 12 and 15. A positional candidate gene on SSC10, *aldo-keto reductase 1C2*, showed an association with age at puberty in the Meishan-White composite resource population [19]. However, the causal gene(s) remain(s) unknown so far.

Erhualian is a Chinese indigenous pig breed that is characterised by early maturity and prolificacy [31], and White Duroc is a commercial composite boar line with good growth performance and a late puberty age. We have developed a White Duroc-Erhualian F<sub>2</sub> resource population. Thus, the objective of this study was to identify QTL for age at puberty in this population using a whole-genome scan.

## 2. MATERIALS AND METHODS

### 2.1. Animals

A three-generation F<sub>2</sub> population was developed by crossing the Chinese Erhualian and the White Duroc pigs. Two White Duroc boars and 17 Chinese Erhualian sows were mated to produce F<sub>1</sub> pigs, from which 9 F<sub>1</sub> males and 59 F<sub>1</sub> females were intercrossed to produce six batches of 1912 F<sub>2</sub> pigs. In this assay, F<sub>2</sub> gilts ( $n = 541$ ) from 55 families were recorded for age at puberty.

## 2.2. Phenotype recording

All gilts were kept in half-open houses, each of which held 10 gilts and 1 castrated boar. During the period between day 90 and day 240, all F<sub>2</sub> gilts were checked twice a day for oestrus signs at approximately 09:00 and 15:30 by inspection of the vulva and detection of the standing reflex using the back pressure test [9]. Oestrus signs including redness, degree of swelling, mucosal discharge from the vulva and the standing reflex during puberty were recorded. All gilts were slaughtered at about 240 days. Their ovaries were then checked to verify historical records of oestrus cycle. Age at puberty was defined as the age at which the first oestrus sign was observed.

## 2.3. Genotyping

Genomic DNA was extracted from pig ear tissues or blood using the standard phenol/chloroform method. All DNA samples were quantified with a DU640 spectrophotometer (Beckman, CA, USA) and diluted to a standardised concentration of 20 ng·μL<sup>-1</sup> in 96-well plates. Microsatellite markers were selected from the USDA-MARC linkage map (<http://www.animalgenome.org/maps/marcmap.html/>) to genotype all founder and F<sub>1</sub> animals of the White Duroc × Erhualian intercross. A final set of 183 informative markers covering 19 porcine chromosomes was used to carry out a whole-genome scan across the entire resource population. The polymerase chain reaction conditions for each marker locus were optimised using standard protocols. After amplification with primers labelled by three fluorescent dyes (NED, FAM and HEX), genotypes were recorded and collected in an ABI PRISM® 3130XL Genetic Analyser with the GeneMapper™ Genotyping Version 3.7 (Applied Biosystems, Foster City, USA).

## 2.4. Statistics

The sex-average linkage map was constructed and analysed using the BUILD and CHROMPIC options of CRI-MAP Version 2.4 [11]. The QTL mapping analysis was performed using the QTLexpress software available at <http://qtl.cap.ed.ac.uk/> [12]. This method is based on the assumption that alternative alleles for a QTL are fixed in the two founder breeds, respectively, and follows a robust two-step procedure to identify QTL. First, the probabilities of alleles for each individual in the F<sub>2</sub> generation were calculated for every centimorgan (cM) throughout the genome using the information of the flanking markers. Second, a multiple linear regression model with the additive (a) and dominance (d) effects of a QTL at a given position and other fixed or random

effects were fitted by least squares for each interesting trait [15]. The following one-QTL model was initially used to detect the primary QTL:

$$\mathbf{Y}_i = \mu + \text{batch}_i + \text{maternal}_k + w21_i + c_{ai}a + c_{di}d + e_i, \quad (1)$$

where  $\mathbf{Y}_i$  is the phenotype of individual  $i$ ,  $\mu$  is the overall mean of the phenotype,  $\text{batch}_i$  is the fixed effect of  $F_2$  batch,  $\text{maternal}_k$  is another fixed effect of the individual  $i$ 's maternal effect,  $w21_i$  is the individual  $i$ 's weight as a covariate,  $a$  and  $d$  are the QTL additive and dominant effects and  $e_i$  is the residual effect. The  $c_{ai}a$  and  $c_{di}d$  are the coefficients of additive and dominant effects, respectively, which were defined as described by de Koning *et al.* [8]. After the primary QTL analysis, the multiple QTL model was used, in which QTL effects, initially detected, were integrated as fixed effects.

The genome-wide significance thresholds were determined by a permutation test as described by Churchill and Doerge [5]. The chromosome-wide significance level was used as the suggestive level, which was inferred from the 5% genome-wide level using:

$$P_{\text{chromosome}} \approx 1 - (1 - P_{\text{genome}})^{19} \quad (2)$$

to correct the multiple tests [7].

### 3. RESULTS

The pubertal stage was confirmed in 454 of the 541  $F_2$  gilts studied by consistent historical records and *in vitro* ovary examination, including 344 cyclic animals and 110 juvenile animals that did not reach puberty at the age of 240 days. The average age at puberty in the cyclic animals was  $192.6 \pm 28.59$  days and ranged from 123 to 240 days, showing diverse phenotypic segregations in the  $F_2$  population. The juvenile animals showing no defect in ovary development were assumed to reach puberty randomly at an age ranging from 240 to 300 days, and were also used for the QTL analysis together with the cyclic animals.

A whole-genome linkage map comprising 183 microsatellite markers was constructed. Its total length was 2350.3 cM and the average marker interval on the sex-average map was 12.84 cM. The marker orders were consistent with the USDA-MARC reference map (data not shown).

We performed the QTL analysis under a multiple QTL model. The  $F$ -ratios of significance thresholds for the 0.1, 1 and 5% genome-wise significance levels and the 1 and 5% chromosome-wise significance levels were 14.0, 9.9, 8.6, 7.0 and 5.2, respectively.

**Table 1.** Positions of QTL for age at puberty in the White Duroc  $\times$  Erhualian intercross.

SSC	Position (cM)	F-value	Additive effect $\pm$ SE	Dominance effect $\pm$ SE	Var%
1	114	9.98	$-10.47 \pm 2.76$	$-7.34 \pm 3.84$	3.9
7	54	19.19	$15.18 \pm 2.47$	$-4.13 \pm 3.52$	8.0
8	77	5.49	$-8.08 \pm 2.44$	$1.29 \pm 3.58$	2.0
17	88	6.61	$-2.56 \pm 2.52$	$-12.41 \pm 3.46$	2.4

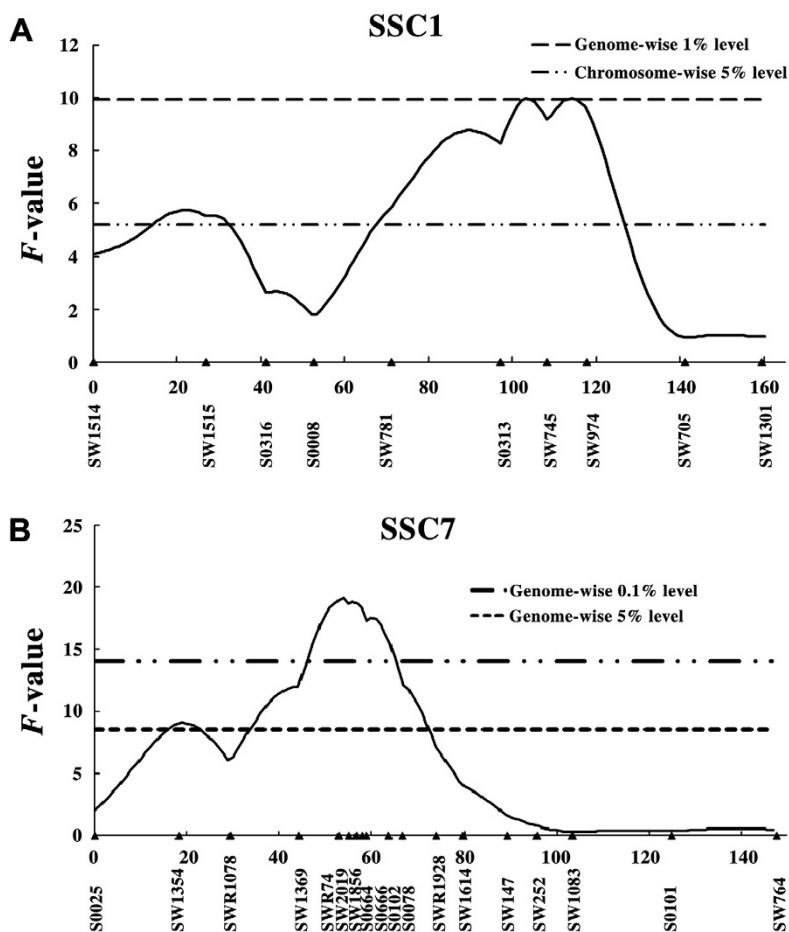
Four significant QTL were found for age at puberty in the White Duroc  $\times$  Erhualian intercross (Tab. 1), and *F*-statistic curves for these QTL are shown in Figure 1. A 1% genome-wide significant QTL was found at position 114 cM on SSC1 (Fig. 1A) and the Erhualian allele at this locus is favourable for decreased age at puberty. A 0.1% genome-wide significant QTL was detected on SSC7 at 54 cM (Fig. 1B) and interestingly, the Duroc allele is favourable for decreased age at puberty at this locus. Two 5% chromosome-wide significant QTL were identified for age at puberty on SSC8 at 77 cM (Fig. 1C) and SSC17 at 88 cM (Fig. 1D), respectively. In these two QTL regions, favourable alleles were observed in the Erhualian breed.

#### 4. DISCUSSION

The resource population used for the QTL analysis in this study was established by crossing the Chinese Erhualian sows and the White Duroc boars. The ages at puberty of Erhualian and White Duroc pigs are 79.2 days [31] and 6–8 months [2], respectively. The remarkable phenotypic and genetic original differences between the grandparents [17] make this resource population suitable to identify genomic regions affecting age at puberty.

Phenotypic definition and assessment are critical issues for QTL mapping, especially for some traits that are difficult to record. In this study, age at puberty of each gilt was confirmed by the following oestrus cycle, and juvenile animals were checked for ovary development when they were slaughtered at the age of 240 days. The mean age at puberty in this  $F_2$  population is greater (192.6 days vs. 181.3 days) than in a resource population of  $F_2$  females reported by Cassidy *et al.* [4]. This is probably due to the fact that these  $F_2$  gilts were checked without boar exposure after 90 days when ages at puberty were measured. It is well known that boar exposure can accelerate sexual development in gilts [3,28].

The timing of puberty in humans approximates a normal or Gaussian distribution [20]. In this study, we found that the phenotype data of cyclic  $F_2$  animals



**Figure 1.** The *F*-ratio test curve indicating QTL for age at puberty on SSC1 (A), SSC7 (B), SSC8 (C) and SSC17 (D). Markers and distances in cM are given on the *x*-axis, and *F*-ratios are indicated on the *y*-axis.

followed a skewed distribution (Fig. 2), and that the ovaries of juvenile  $F_2$  animals were normally developed when slaughtered at the age of 240 days (data not shown). The phenotype data of  $F_2$  animals conformed to the Gaussian distribution when age at puberty of each juvenile animal was randomly assigned in a range of 240–300 days. Thus, it is reasonable to assume that juvenile animals with a normal ovary development generally reach puberty after the age of 240–300 days. We then performed the QTL analysis using combined data of cyclic animals and juvenile animals and found evidence for a genomic region affecting age at puberty.

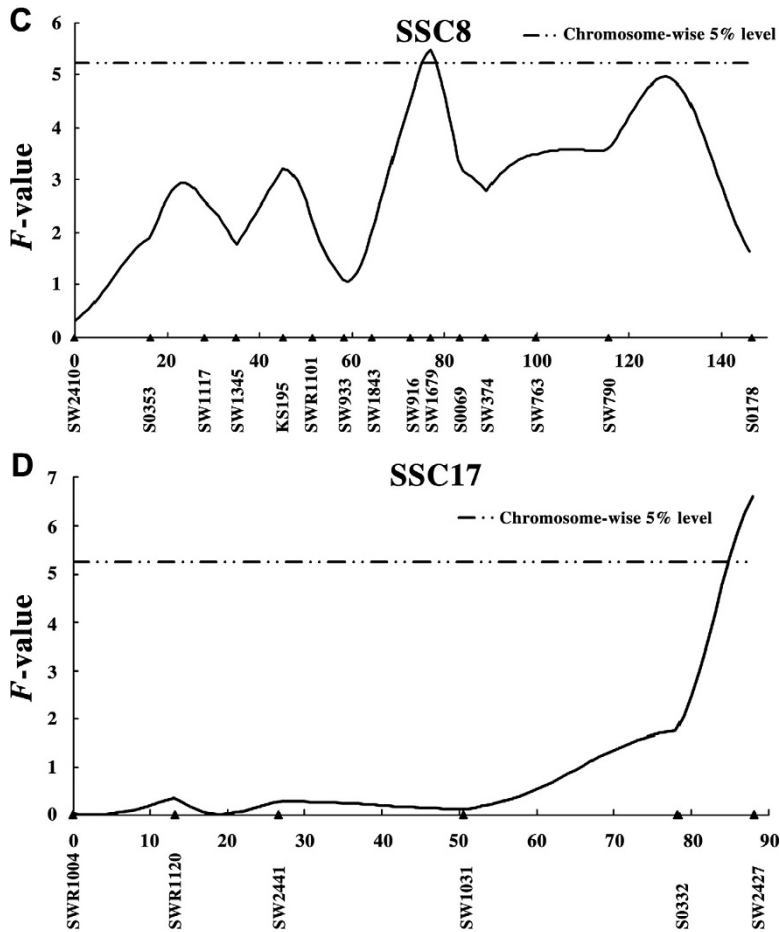
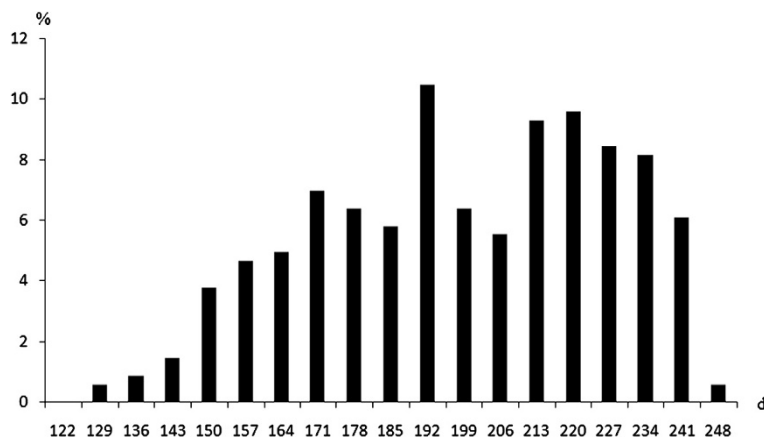


Figure 1. Continued.

Until now, only a limited number of QTL for age at puberty have been identified in pigs (<http://www.animalgenome.org/QTLdb/pig.html>). We found a 0.1% genome-wide significant QTL for age at puberty at position 54 cM on SSC7 close to the SLA complex, which is the most significant QTL found in this study, confirming previous QTL mapping results [4,14]. SSC7 is of particular interest to the pig industry since it carries numerous QTL affecting diverse economically important traits. At the QTL for age at puberty on SSC7, one interesting phenomenon is that the favourable QTL allele originates from the Duroc breed, which is in contradiction with the breed characteristic differences for this trait. This observation has been repeatedly documented in many QTL studies around this region, such as the QTL for fat androstenone levels [21] and for



**Figure 2.** The distribution of age at puberty in the  $F_2$  population of the White Duroc  $\times$  Erhualian intercross.

average daily gain [26]. The reason for this remains unknown. Although many QTL have been evidenced in the region around the SLA complex, it is difficult to know whether these QTL are due to the pleiotropic effect of a single locus or to effects of closely linked genes because of high gene density in the region.

We confirmed the existence of a QTL at 114 cM for age at puberty on SSC1 that had been previously found in the crossbred Meishan-White composite population [24]. Several significant QTL for average daily gain and average backfat thickness have been mapped at a position close to the QTL region found in this study [6,18]. It has been shown that age at puberty shows high genetic correlations with body weight [30], growth rate and fatness in pigs [10,25]. The overlapping QTL region for age at puberty, growth and fatness traits indicates that there might be gene(s) with pleiotropic effects on these traits in the region.

Another QTL for age at puberty on SSC8 was consistently detected by our research group and others [4,14]. The QTL is centred at 77 cM, where QTL for litter size [16], ovulation rate [22], uterine capacity [24] and number of nipples [1] have been found. The multiple associations with these correlated reproductive traits displayed convincing evidence for the QTL for age at puberty in this region.

A suggestive QTL for age at puberty was detected at 88 cM on SSC17. To our knowledge, this is the first time that this QTL region is reported. However, we did not detect the QTL on SSC10, 12 and 15 that were previously reported. These differences are possibly due to the different genetic backgrounds of founder animals in different experimental populations. It highlights that the



implementation of the MAS program in different populations using QTL markers requires caution.

In conclusion, we found a novel QTL for age at puberty on SSC17 but with a low significance level and confirmed those on SSC1, 7 and 8. The two QTL regions on SSC7 and SSC1 are of particular interest, showing highly significant associations across different resource populations. Future work will be directed at fine mapping of the QTL identified using additional markers and advanced intercross populations or recombination backcross populations [26].

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